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Research Note—

Passive West Nile Virus Antibody Transfer from Maternal Eastern Screech-Owls (*Megascops asio*) to Progeny

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SUMMARY. Transovarial antibody transfer in owls has not been demonstrated for West Nile virus (WNV). We sampled chicks from captive adult WNV-antibody-positive Eastern Screech-Owls (*Megascops asio*) to evaluate the prevalence of transovarial maternal antibody transfer, as well as titers and duration of maternal antibodies. Twenty-four owlets aged 1 to 27 days old circulated detectable antibodies with neutralizing antibody titers ranging from 20 to 1600 (median 1:40). Demonstrating that WNV antibodies are passively transferred transovarially is important for accurate interpretation of serologic data from young birds.

RESUMEN. *Nota de Investigación*—Transferencia pasiva a la progenie del búho chillón oriental (*Megascops asio*), de anticuerpos maternales contra el virus de oeste del Nilo.

La transferencia transovárica de anticuerpos contra el virus del oeste de Nilo no ha sido demostrada. Con la finalidad de evaluar la prevalencia de la transferencia transovárica de anticuerpos, así como los títulos y la duración de los anticuerpos maternales, se muestrearon polluelos provenientes de búhos chillones orientales (*Megascops asio*) adultos cautivos, positivos a anticuerpos contra el virus del oeste del Nilo. En 24 polluelos de búho de entre 1 y 27 días de edad se detectaron anticuerpos circulantes con títulos de anticuerpos neutralizantes en un rango desde 20 hasta 1600 (promedio de 40). La demostración de que los anticuerpos contra el virus del oeste del Nilo se transfieren transováricamente es importante para la adecuada interpretación de los datos de serología provenientes de aves jóvenes.

Key words: West Nile virus, flavivirus, bird, Eastern Screech-Owl, maternal antibody, passive transfer

Abbreviations: $PRNT_{90}$ = endpoint 90% neutralization level; SLEV = Saint Louis encephalitis virus; WNV = West Nile virus

West Nile virus (WNV; *Flavivirus*, *Flaviviridae*) is an emerging pathogen of public health and veterinary importance. In North America, WNV has been associated with mortality of more than 198 species of birds, including at least 13 species of owls (4,8). However, many owl species are known to survive WNV infection (2,5,7,8,9). Transovarial antibody transfer in birds has not been definitively demonstrated for WNV, although experimental studies in sparrows demonstrated efficient transfer of maternal antibodies and their duration of 3–4 wk for St. Louis encephalitis virus (SLEV), a closely related flavivirus (6). Gibbs *et al.* demonstrated similar duration of passively transferred maternal antibodies to WNV in pigeons (*Columba livia*), but could not rule out crop milk as the means of transfer (3). Crop milk–derived maternal antibody is unique to Columbiformes. For an unambiguous test of transovarial maternal antibody transfer, we sampled chicks from captive adult Eastern Screech-Owls (*Megascops asio*) of known WNV infection history to evaluate the prevalence, titers, and duration of these antibodies.

MATERIALS AND METHODS

Owls in this study were from a captive breeding colony maintained at the U.S. Geological Survey's Patuxent Wildlife Research Center, Laurel, Prince George's County, MD. Some adults in the owl colony had been naturally exposed to WNV. Between April 23 and May 27 of 2004, 10 family groups and additional pens of owlets were sampled. A total of 81 owls were tested, comprising 16 adult owls and 65 owlets (three of which were sampled twice at different ages). Blood samples were drawn from the jugular vein, diluted in saline 10-fold or greater, and clarified by centrifugation. Diluted serum was separated from packed cells and stored at −20 C. Sera were tested for WNV-neutralizing antibodies by the plaque-reduction neutralization test (1). Cross-reactivity for a closely related North American flavivirus, SLEV, was ruled out by comparing 90% neutralization titers ($PRNT_{90}$). A fourfold greater titer of one virus over the other indicated that particular virus as the etiological agent for the infection. We used a conservative set of criteria for characterizing a positive test result to avoid false positive results. $PRNT_{90}$ titers $<$1:20 were considered negative and positives required a $PRNT_{90}$ titer of $\geq$1:20.

RESULTS

Adult owls and their chicks from 10 family groups were sampled. Of these, seven adult females were seropositive, with WNV $PRNT_{90}$ reciprocal titers ranging from 40 to 3200. Seventeen owlets from five of these families also circulated detectable antibodies (Table 1). The lowest titer detectable was 1:20. Titers $\geq$1:20 were considered positive, so low-titered samples may have been falsely classified as negative. Ages of seropositive owlets ranged from 1 to 27 days after hatching, with a maximum titer of 1:1600 (median 1:40). In two cases, all owlets from a seropositive mother were also seropositive, but in other families, 50%–66% of chicks were seropositive, with negative chicks in these cases ranging from 4–16 days of age. In two families, seronegative females and seropositive males produced all seronegative chicks. In one family, owlets were serially sampled between 3–4 and 7–8 days posthatch; antibody levels dropped

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Table 1. Five family groups of Eastern Screech-Owls in which maternal antibody to West Nile virus was passively transferred to progeny.[A]

Family	Bird ID	Gender	Age (days)	$PRNT_{90}$
275	659	M	Adult	40
	667	F	Adult	800
	15	U	4	<20
	79	U	12	<20
	80	U	12	20
	109	U	12	20
	126	U	13	40
	164	U	16	<20
278	620	M	Adult	1600
	622	F	Adult	1600
	162	U	10	20
	161	U	11	40
	163	U	11	60
282	654	F	Adult	1600
	131	U	9	80
	125	U	9	80
285	666	F	Adult	80
	152	U	13	<120
	153	U	17	120
	151	U	18	120
286	591	F	Adult	800
	5	U	1	80
	10[B]	U	3	1600
	10	U	7	40
	11[B]	U	4	800
	11	U	8	40
	20[B]	U	4	100
	20	U	8	20

[A]$PRNT_{90}$ = endpoint 90% neutralization level; M = male; F = female; U = unidentified.

[B]Chicks serially sampled over time, as indicated by ages.

substantially after the initial sampling (Table 1). Among seropositive owl chicks ($n = 24$), age and WNV-specific antibody titer were negatively correlated, using Gamma correlation (gamma value = –0.385, Z = –2.365, $P = 0.02$).

DISCUSSION

An understanding of passive transfer of maternal WNV antibody from adult birds to their progeny is important because of its potential to influence the natural history of WNV transmission, and its potential to interfere with accurate interpretation of field-collected data from neonatal and juvenile birds. We show that seropositive adult owls passively transfer maternal antibody *via* the egg to their progeny. We believe that serum antibodies in chicks aged 1 day to 27 days were maternally derived rather than due to a response to infection, because Great Horned Owls (*Bubo virginianus*) require 6–7 days following infection to produce detectable antibodies to WNV(7), and experimentally infected Eastern Screech-Owls produced detectable antibodies by 7 days postinfection (8). Furthermore, surveillance in Prince George's County, Maryland, failed to detect any WNV activity in birds, horses, people, or mosquitoes until July 2004 (B. Pagac, pers. comm.). The titers that we observed in the owlets were generally higher than previously reported for pigeons (3). Because we did not serially sample all of the owlets in our study over a sufficient time period, we were unable to document the duration of detectable maternal antibody beyond 27 days. Although we did not challenge any of these owlets with WNV infection, we presumed that owlets circulating neutralizing antibody in the peripheral blood would be protected from infection.

Maternal WNV antibody transfer has only been reported for pigeons (3). Five squabs born to naturally infected, captive-housed pigeons demonstrated neutralizing antibody ($PRNT_{90}$) titers ranging from 1:10 to 1:40 with duration of detectable antibody lasting from 19–33 days posthatch. The report did not indicate the antibody titers of the parents, and discussed the possibility of parental transfer of antibody from crop milk in pigeons. The number of seronegative squabs derived from seropositive pigeons was not reported.

In our study, antibody titers among adults and their offspring varied. Variation in antibody titers is inherent to the titration process, and could account for one case of a chick's antibody titer being twofold greater than that of its mother. We are not sure why some chicks of seropositive mothers were seronegative; it is possible that there is a range of antibody titers in chicks from seropositive hens, that maternal antibody levels decay more rapidly in some chicks than others, or that our stringent criteria for a positive result led to false negatives. Additional studies are needed to determine the prevalence of maternal antibody transfer, the range of titers in chicks, the rate of decay of these antibodies, and the duration of protection from WNV infection. Demonstrating that WNV antibodies are passively transferred is important for accurate interpretation of serologic data from young birds.

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